

# Codling moth granulovirus: Variations in the susceptibility of local codling moth populations

Eva Fritsch<sup>1</sup>, Karin Undorf-Spahn<sup>1</sup>, Jutta Kienzle<sup>2</sup>, Claus P.W. Zebitz<sup>2</sup>, Jürg Huber<sup>1</sup>

## Abstract

This study is part of a BMELV (German Federal Ministry for, Food, Agriculture and Consumer Protection) project for prevention of codling moth damage by long-term population control in large areas. Specimens from local codling moth populations were collected in fall 2003 from three different orchards in the South of Germany; two of them having been treated with granulovirus of codling moth (*CpGV* Madex 2 and/or Granupom) over many years (Lake Constance II and South Baden) and one since two years (Lake Constance I). In autumn 2004, specimens from populations in four other orchards with serious CM problems were collected to investigate whether more populations were involved in that region. Furthermore, the population Lake Constance I and South Baden were tested again. During the season, the location South Baden was almost weekly treated with 100 ml/ha of Madex 2 to test whether the level of susceptibility would change after such heavy treatments.

The susceptibility of the offsprings of the overwintering generation to *CpGV* was investigated in the spring of the following year in bioassays on artificial diet and compared to a laboratory strain of the codling moth. The results indicated significant differences in sensitivity to the virus between the local codling moth populations. The  $LC_{50}$ -values showed that in 2004, South Baden and Lake Constance II were more than thousand fold less susceptible than the populations Lake Constance I and the laboratory strain. The results from the bioassays in 2005 confirmed the low susceptibility of South Baden and of the new locations in Saarland and from an orchard 100 km away from South Baden. The population Lake Constance I, on the other hand, maintained its high sensitivity to the virus. The slope of the dose-mortality-regression lines of the unsusceptible populations was significantly lower than those of the susceptible ones, including that of the laboratory strain. This indicates a high inhomogeneity in the individual response of the unsusceptible larvae against the virus. Actually, the problem of reduced sensitivity to the virus seems to be limited to a few orchards in Germany, the majority of orchards being not affected.

**Keywords:** *CpGV*, granulovirus, baculovirus, codling moth, susceptibility, field population, field tests, integrated plant protection

## Introduction

Background of this study was a project on prevention of codling moth damage by long-term population control in wide areas using codling moth granulovirus (*CpGV*), funded by BMELV (German Federal Ministry for Food, Agriculture and Consumer Protection). The codling moth granulovirus (*CpGV*) is applied in ecologically treated apple orchards in Germany for more than ten years. It is used not only for direct reduction of fruit damage but also to reduce the population density of the subsequent codling moth generation. All the commercially available *CpGV*s are based on only one genotype, the so-called "Mexican isolate". Though, due to the biological characteristics of baculoviruses the development of resistance is assumed rather unlikely (Granados & Federici, 1986) and, so far, there were no reports of resistance of codling moth against *CpGV* in the field. In the framework of the BMELV study, the possible presence of resistance to *GpGV* in some CM populations was monitored. Since 2002, one apple grower in South Baden started to observe high codling moth infestation in spite of repeated *CpGV* applications. To find out whether this phenomenon is based on a reduced susceptibility of the codling moth, individuals of this local population in South Baden (planted in fall 1996 on farmland and treated since then with *CpGV*) and also of two other populations from the area of Lake Constance (I: IP, treated over 2 years and II: organic, planted 1992 on

<sup>1</sup> BBA, Institute for Biological Control, Heinrichstr. 243, D-64287 Darmstadt, Germany;

<sup>2</sup> University of Hohenheim, Institute of Phytomedicine, D-70593 Stuttgart, Germany

grassland and treated since then with *CpGV*) were collected in 2003. In 2004, some additional orchards from other locations were included in the study. The offsprings of the overwintering generations were examined in bioassays on artificial diet in the laboratory and compared to a laboratory strain of the codling moth serving as a standard.

## **Material and Methods**

### Virus

The granulovirus of the codling moth used in the bioassays is a descendent from the *CpGV* collected in Northern Mexico ("Mexican strain") (Tanada, 1964). It was propagated in host insects and purified by the method described by Huber (1981).

### Test insects

Wild codling moth larvae were collected with corrugated cardboard bands placed around the trunk of apple trees in the respective orchards and maintained in a frost-free room during winter. In spring they were kept on a sheltered balcony until hatch of the moths at the beginning of May. The moths were kept at room temperature and under natural light conditions in rearing containers for copulation and oviposition.

Larvae of a laboratory strain of the codling moth served as standard in the bioassays. This laboratory strain has been established more than thirty years ago at the Institute for Biological Control of the BBA in Darmstadt. The rearing method has been described by Bathon (1981).

### Bioassay method

The bioassays were conducted following the method described by Huber (1981). The virus was incorporated into an artificial diet by thorough mixing of *CpGV* suspensions of different concentrations with codling moth diet (Ivaldi-Sender, 1974) kept at a temperature of 45 °C in a water bath. The mixture was dispensed into special boxes (LICEFA, Bad-Sulzuflen, Germany) with 50 separate cells (1.5 x 1.5 x 2 cm). One neonate larva was placed in each cell. The boxes were covered with a layer of tissue paper, a polyethylen sheet, and a hard-plastic cover, and fixed with two rubber bands. The boxes were incubated at 26 °C, 60-70 % RH with an 16 hr photoperiod. Larvae were examined for virus mortality 6 days and 14 days after start of the bioassay. Concentration - larval mortality – correlation was calculated using a probit analysis programme based on a maximum likelihood procedure (MLP 3.08, NAG, Lawes Agricultural Trust, Rothamstead Experimental Station, 1987) in order to obtain LC<sub>50</sub> values and other parameters of the dose mortality response.

## **Results and discussion**

### Susceptibility of codling moth strains collected in autumn 2003

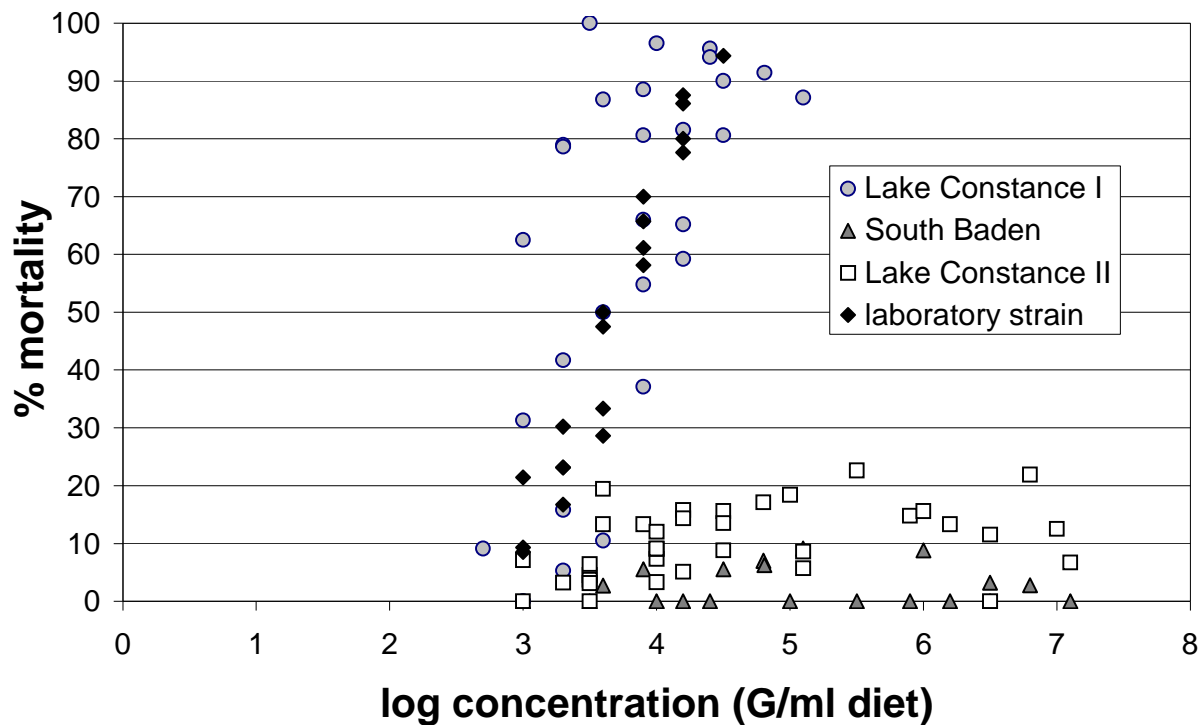
The neonate offsprings (F1-generation) of the diapausing generation from the three local field populations (Lake Constance I., Lake Constance II and South Baden) were submitted to a bioassay to estimate their susceptibility for granulovirus. The data were compared with the mortality obtained with a codling moth laboratory strain, showing a normal susceptibility for the *CpGV*.

First results from bioassays evaluated after 6 days indicated significantly lower mortality for the strains Lake Constance II and South Baden than for the strain Lake Constance I (Figure 1). Even with increased virus concentrations of up to  $1 \times 10^7$  G/ml medium, larval mortality did not exceed 20 %. In comparison, the sensitivity of the strain Lake Constance I was similar to that observed for the

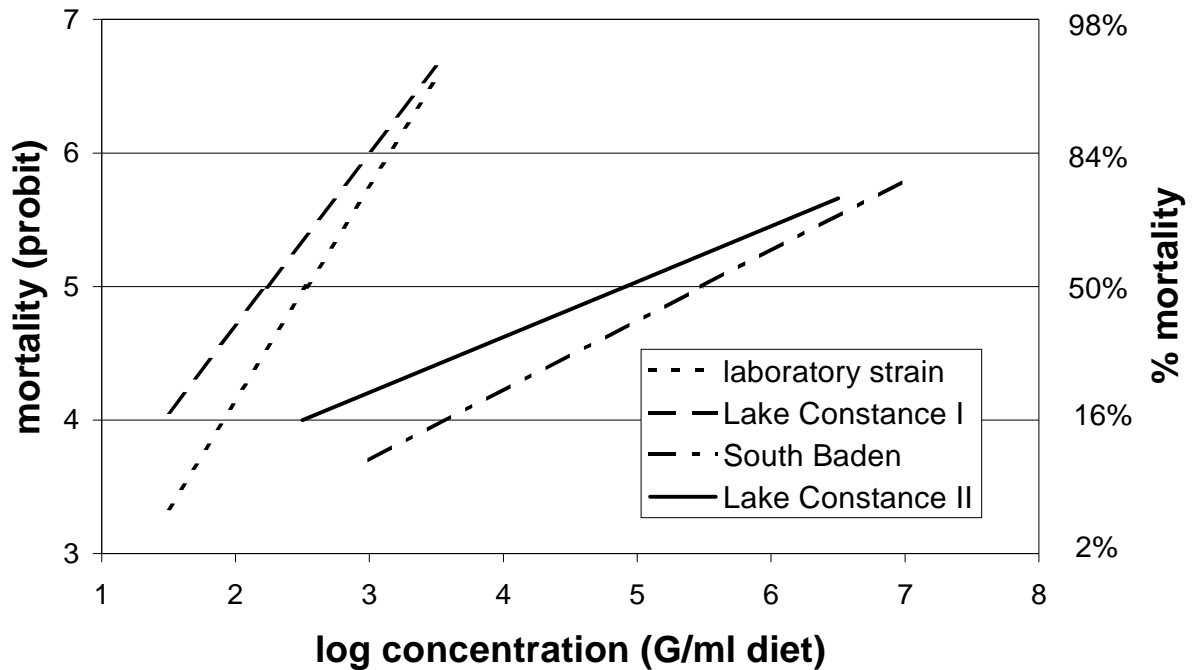
laboratory strain. Larval mortality raised from 10 % to 100 % with an increase in virus concentration from  $1 \times 10^3$  G/ml medium to  $1 \times 10^4$  G/ml.

To find out whether the two less susceptible populations (Lake Constance II and South Baden) are able to survive high virus concentrations also for a longer time, the test insects were kept over 14 days. This resulted in higher mortality. However, even larvae exposed to the dose of  $10^7$  G/ml medium showed 85 % mortality only.

For an accurate determination of differences between the codling moth strains in their susceptibility to the virus, mortality data obtained from bioassays were subjected to probit analysis (Table 1). Important differences in the slope of the regression lines (Figure 2) were detected, indicating heterogeneity of the insect populations towards the virus. The slope of the regression line of Lake Constance I, which corresponded very well to the regression line of the normal, sensitive laboratory strain, was steeper (slope 1.3) than that for the two others, Lake Constance II (slope 0.4) and South Baden (slope 0.5). In addition,  $LC_{50}$  values calculated by probit analysis (Table 1) differed significantly between some of the four codling moth populations.



**Figure 1.** Mortality data obtained from bioassays with different codling moth strains (collected in autumn 2003) 6 days after incubation. Each symbol represents the mortality recorded from 50 test insects.



**Figure 2.** Probit regression lines of the different codling moth populations (collected in autumn 2003) obtained from bioassays 14 days after incubation.

In a comparison of the data obtained 6 days after start of experiments between the strain Lake Constance I and the laboratory strain, no significant differences in the  $LC_{50}$  values ( $8,6 \times 10^3$  and  $5,1 \times 10^3$  G/ml) were found (Chi Square Test,  $p = 95\%$ ). Bioassays for the two strains incubated for 14 days provided also dose response curves with significant parallelism and comparable  $LC_{50}$  values (169 and 341 G/ml). Compared to the field strain Lake Constance I, the  $LC_{50}$  values of the strains South Baden and Lake Constance II were significantly increased by a factor of 1,000 and of 500, respectively (Table 1). This indicates that the susceptibility for the CpGV is highly reduced in these two field populations.

These results obtained from bioassays confirmed that the local codling moth field populations differ in their susceptibility to the CpGV. The F1 generation originating from the orchards South Baden and Lake Constance II were significantly less sensitive than the strain from the orchard Lake Constance I. These differences in susceptibility are represented by  $LC_{50}$  values being approximately 1,000 times higher than for the normal sensitive strain Lake Constance I. In addition, the lower slope of the probit regression lines indicates a higher variation in susceptibility among individuals of the populations South Baden and Lake Constance II. The individuals of Lake Constance I showed to be as sensitive as those of the laboratory strain, which is indicated by similar  $LC_{50}$  values and almost the same steep dose response regression lines.

**Table 1.** Susceptibility of different codling moth strains, collected in fall 2003 and evaluated in bioassays with neonates after 6 days and 14 days incubation. The slopes of the probit mortality regression lines and the calculated  $LC_{50}$  values are listed.

codling moth strain / location	incubation time	$LC_{50}$ [G/ml diet] (95% conf. limits)	Slope (standard error)
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laboratory strain	6 days	$5.1 \times 10^3$ (4.48 – 5.82)	1.77 (0.126)
	14 days	$3.41 \times 10^2$ (2.74 – 4.41)	1.61 (0.186)
Lake Constance I	6 days	$8.57 \times 10^3$ (6.87 – 10.76)	1.49 (0.130)
	14 days	$1.69 \times 10^2$ (1.05 – 2.49)	1.29 (0.129)
South Baden	6 days	— <sup>*</sup>	— <sup>*</sup>
	14 days	$3.02 \times 10^5$ (2.07 – 4.62)	0.52 (0.041)
Lake Constance II	6 days	— <sup>*</sup>	— <sup>*</sup>
	14 days	$8.16 \times 10^4$ (5.09 – 13.80)	0.41 (0.037)

\* Due to the low mortality rates no significant regression lines could be estimated.

### Susceptibility of codling moth strains collected in autumn 2004

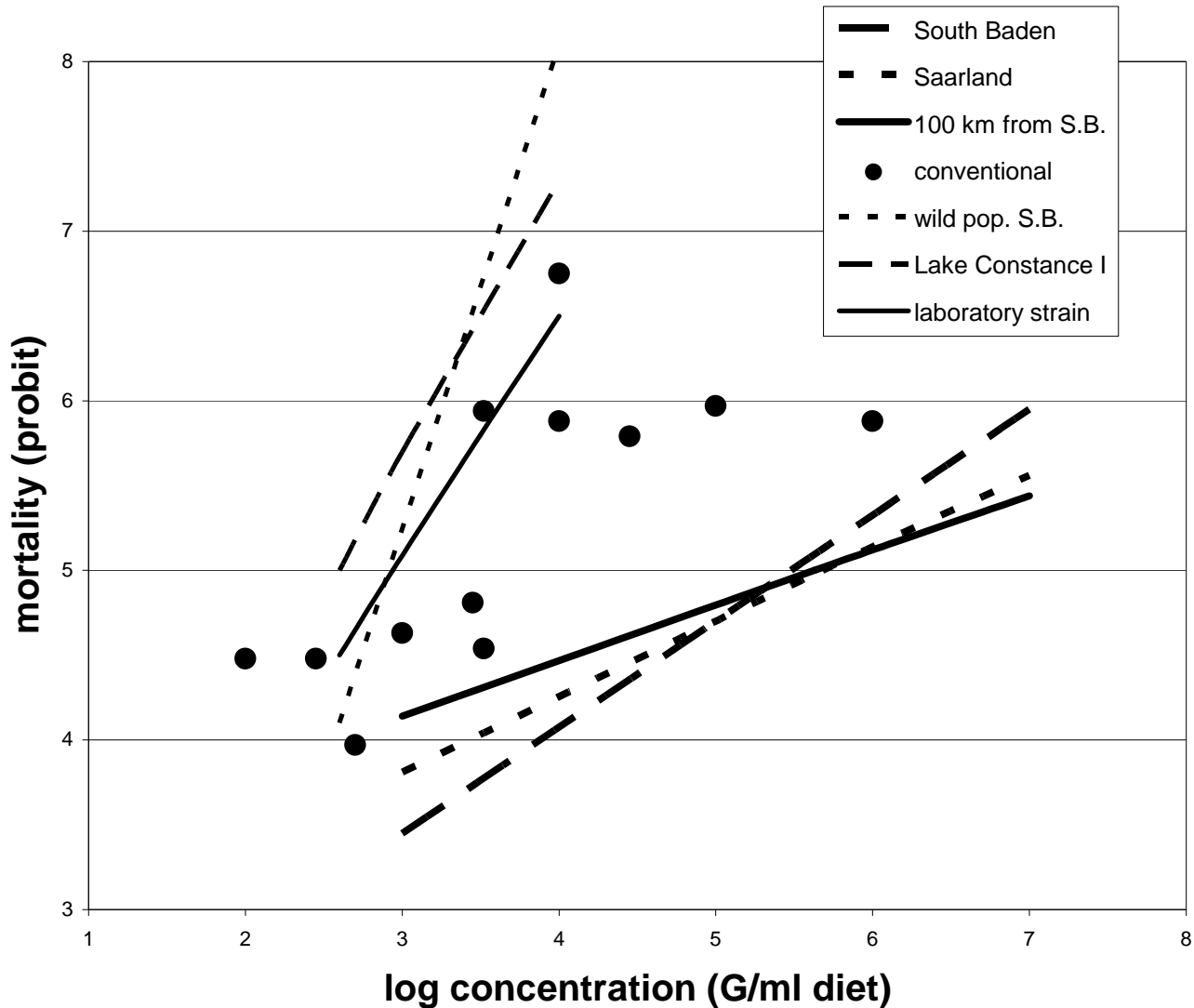
In autumn 2004, the specimens of the populations South Baden and Lake Constance I were collected again as well as from four populations from other orchards (one organic orchard in Saarland, one conventional orchard near Lake Constance, one organic orchard 100 km from location South Baden and one wild population near South Baden). The offsprings of these overwintering larvae were tested for their susceptibility to the granulovirus using 14-days bioassays. The dose-mortality regression lines and the calculated LC<sub>50</sub>-values are presented in Figure 3 and Table 2.

The results from the bioassays 2005 confirmed again the low susceptibility of the population South Baden. Although this population was heavily treated with CpGV during the season in 2004 the degree of susceptibility did not change in comparison to the year before. On the other hand, the population Lake Constance I maintained its high sensitivity to the granulovirus. The slopes of the dose-regression lines and the LC<sub>50</sub>-values corresponded very well to the results obtained in 2004. Furthermore the wild population South Baden proved to be as susceptible as the laboratory strain. The other tested populations showed significantly lower slopes of the dose-response regression lines and higher LC<sub>50</sub>-values than those of the more susceptible strains (Lake Constance I and the wild population South Baden). Again, very high virus concentrations up to 10<sup>7</sup> G/ml in the bioassay diet were not capable to kill more than 84 % (probit 6) of the test insects. The estimated LC<sub>50</sub>-values increased by a factor of nearly 1000 in comparison to the susceptible strains. The results of the bioassays with the codling moth larvae originating from the conventional orchard Lake Constance indicated that the susceptibility of this population is between the most sensitive and most resistant strains. To demonstrate this finding, in Figure 3, the individual mortality data of the bioassays are plotted against the virus concentrations. The mortality response of some of the individuals increased linear with the virus concentration in a range of 10<sup>2</sup> to 10<sup>5</sup> G/ml diet. For the remaining insects, a further increase of virus concentration up to 10<sup>7</sup> G/ml diet did not raise the mortality to more than 85%. This indicates a high

inhomogeneity in the individual response of these larvae against the virus. It looked as if 95 % of the larvae had normal sensitivity for the virus, whereas 15 % were (already) unsusceptible.

**Table 2.** Susceptibility of the different codling moth strains, collected in fall 2004 and evaluated in bioassays with neonates after 14 days incubation.

<b>codling moth strain / location</b>	<b>LC<sub>50</sub>[G/ml diet] (95% conf. limits)</b>	<b>slope (standard error)</b>
laboratory strain	$8.77 \times 10^2$ (7.57 – 10.25)	1.39 (0.112)
Lake Constance I	$3.94 \times 10^2$ (3.16 – 5.00)	1.67 (0.16)
Wild-population near location South Baden	$8.01 \times 10^2$ (6.33 – 11.66)	2.79 (0.638)
South Baden, after another year of intensive virus application	$3.05 \times 10^5$ (1.93 – 4.70)	0.62 (0.046)
organic orchard with 40 % CM infestation, 100 km distance to location South Baden	$4.31 \times 10^5$ (1.71 – 12.70)	0.33 (0.045)
organic orchard with 80 % CM infestation, Saarland	$4.79 \times 10^5$ (1.75 – 2.30)	0.44 (0.071)
conventional orchard, virus-use never fully satisfying, Lake Constance	$2.02 \times 10^3$ (1.04 – 3.59)	0.53 (0.065)



**Figure 3.** Comparison of the dose-mortality data of the conventional treated strain Lake Constance with the regression lines of the other populations collected in autumn 2004.

At present it is not possible to decide whether the reduced susceptibility is a result of a selection process or whether these less sensitive populations already existed in the orchards before the beginning of the virus applications. Also an immigration of unsusceptible codling moth individuals into the *CpGV* treated apple orchards should be considered.

In recent years, *CpGV* has been applied in Europe in a large scale, however, the phenomenon of a reduced sensitivity of field populations has not been observed yet. The described problem of reduced sensitivity to the virus seems to be limited to a few orchards in Germany whereas the majority of orchards are not affected.

There are several reports about insect strains becoming resistant against insect viruses under heavy selection pressure in the laboratory. Fuxa and Richter (1998), e.g., were able to induce resistance to a nucleopolyhedrovirus in a laboratory strain of *Spodoptera frugiperda*. From the field, no cases of resistance have been reported, so far.

For the present case, it might be of importance that in the field only one genotype of *CpGV* (Mexican strain) was applied during all the years. The continued exposure of rather heterogeneous host populations to a homogenous virus may have favoured the development of resistance.

A conclusion on the significance of these first observations for the practical use of the granulovirus is not possible without more data about the distribution of the phenomenon in German and European orchards being available. In addition, the examination of the genetic background of different populations and studies on possible mechanisms of resistance have to be initiated.

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